Available online at www.sciencedirect.com
ScienceDirect
Journal of Insect Physiology 53 (2007) 113–125
Journal of Insect Physiology
www.elsevier.com/locate/jinsphys

Acclimation effects on thermal tolerances of springtails from sub-Antarctic Marion Island: Indigenous and invasive species

Sarette Slabber[a], M. Roger Worland[b], Hans Petter Leinaas[c], Steven L. Chown[a,*]

[a]*Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, South Africa*
[b]*British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge CB3 0ET, UK*
[c]*Programme for Experimental Behavioural and Population Ecology Research, Department of Biology, University of Oslo, Norway*

Received 26 July 2006; received in revised form 25 October 2006; accepted 25 October 2006

Abstract

Collembola are abundant and functionally significant arthropods in sub-Antarctic terrestrial ecosystems, and their importance has increased as a consequence of the many invasive alien species that have been introduced to the region. It has also been predicted that current and future climate change will favour alien over indigenous species as a consequence of more favourable responses to warming in the former. It is therefore surprising that little is known about the environmental physiology of sub-Antarctic springtails and that few studies have explicitly tested the hypothesis that invasive species will outperform indigenous ones under warmer conditions. Here we present thermal tolerance data on three invasive (*Pogonognathellus flavescens*, *Isotomurus cf. palustris*, *Ceratophysella denticulata*) and two indigenous (*Cryptopygus antarcticus*, *Tullbergia bisetosa*) species of springtails from Marion Island, explicitly testing the idea that consistent differences exist between the indigenous and invasive species both in their absolute limits and the ways in which they respond to acclimation (at temperatures from 0 to 20 °C). Phenotypic plasticity is the first in a series of ways in which organisms might respond to altered environments. Using a poorly explored, but highly appropriate technique, we demonstrate that in these species the crystallization temperature (T_c) is equal to the lower lethal temperature. We also show that cooling rate (1 °C min^{-1}; 0.1 °C min^{-1}; 0.5 °C h^{-1} from 5 to −1 °C followed by 0.1 °C min^{-1}) has little effect on T_c. The indigenous species typically have low T_cs (c. −20 to −13 °C depending on the acclimation temperature), whilst those of the invasive species tend to be higher (c. −12 to −6 °C) at the lower acclimation temperatures. However, *Ceratophysella denticulata* is an exception with a low T_c (c. −20 to −18 °C), and in *P. flavescens* acclimation to 20 °C results in a pronounced decline in T_c. In general, the invasive and alien species do not differ substantially in acclimation effects on T_c (with the exception of the strong response in *P. flavescens*). Upper lethal temperatures (ULT50) are typically higher in the invasive (33–37 °C) than in the indigenous (30–33 °C) species and the response to acclimation differs among the two groups. The indigenous species show either a weak response to acclimation or ULT50 declines with increasing acclimation temperature, whereas in the invasive species ULT50 increases with acclimation temperature. These findings support the hypothesis that many invasive species will be favoured by climate change (warming and drying) at Marion Island. Moreover, manipulative field experiments have shown abundance changes in the indigenous and invasive springtail species in the direction predicted by the physiological data.

Keywords: Cold tolerance; Phenotypic plasticity; Supercooling point; Upper thermal limits

1. Introduction

Springtails form an important component of the Antarctic terrestrial arthropod fauna. Together with the mites they are the only arthropods that occupy ice-free land in the continental Antarctic and are absent from only a few such areas (Wise, 1967; Block, 1984; Ryan and Watkins, 1989; Convey and McInnes, 2005; Sinclair et al., 2006). Further north, despite the increase in diversity of true insects (Chown et al., 1998; Chown and Convey, 2007), springtails continue to play an important role in terrestrial systems given their high diversity and exceptional abundance (Block, 1982a; Sømme, 1986a; Usher and Booth, 1986; Greenslade, 1990; Convey et al., 1999, 2000). Moreover, on the sub-Antarctic islands the richness of

*Corresponding author. Tel.: +27 21 808 2385; fax: +2721 808 2405.
E-mail address: slchown@sun.ac.za (S.L. Chown).

0022-1910/$ - see front matter © 2006 Published by Elsevier Ltd.
doi:10.1016/j.jinsphys.2006.10.010

indigenous species has been augmented by the invasion of several, mostly European, springtail species, which can reach exceptional abundances in lowland communities (Convey et al., 1999; Gabriel et al., 2001; Frenot et al., 2005). Indeed, it is suspected that these invasive species may well have displaced indigenous ones on at least some islands (e.g. Convey et al., 1999).

Given their importance in terrestrial systems the continental and maritime Antarctic springtail fauna has enjoyed considerable research attention, especially from the perspective of their environmental physiology. One species, the isotomid *Cryptopygus antarcticus*, has been especially well investigated (e.g. Sømme, 1978; Sømme and Block, 1982; Cannon, 1986; Harrisson et al., 1991; Hayward et al., 2001; Worland and Convey, 2001), but considerable attention has also been given to a variety of other species (e.g. Sinclair and Sjursen, 2001; Sinclair et al., 2003a, 2006). By contrast, the sub-Antarctic springtails have been poorly investigated from this perspective. Indeed, with the exception of three studies no investigations of the environmental physiology of springtails from this region have been undertaken. These exceptions are a thorough study of the factors affecting the freezing of *Tullbergia antarctica* from the Kerguelen Islands (Worland, 2005), another of the effects of acclimation on haemolymph osmolality and thermal hysteresis of one indigenous and two introduced springtail species from Marion Island (Sinclair and Chown, 2002), and a third of the influence of moulting on freezing in an invasive species (Worland et al., 2006).

This situation is remarkable for several reasons, of which two are most significant. First, broad-scale ecological studies have shown that the indigenous springtail species on Marion Island are more abundant in cooler, high altitude habitats whereas invasive species are more abundant in warmer lowland ones (Gabriel et al., 2001), and a similar distribution of indigenous and invasive species seems to be characteristic of South Georgia (Convey et al., 1999). Even on the small scale of individual cushion plants occurring in fellfield, indigenous springtails on Marion Island appear to prefer the cooler parts of the cushions (Hugo et al., 2004). This suggests that the indigenous springtails might be less tolerant of high temperatures than the invasive species (either absolutely or relatively), and vice versa for low temperatures. Second, the climates of many of the sub-Antarctic islands are warming and drying in step with global climate change (Bergstrom and Chown, 1999). For example, at Marion Island mean annual temperature has increased by more than 1 °C and total annual precipitation has declined by more than 500 mm over the past 50 years, and the trend is predicted to continue (Smith, 2002). In the context of the ecological data on the springtails at the island this suggests that in the future the indigenous species will be at a disadvantage in comparison with the invasive ones. Recent manipulative experiments have borne out this prediction (McGeoch et al., 2006), which also matches an expectation expressed more broadly in the literature that with global climate change invasive species will be favoured at the expense of indigenous ones (Dukes and Mooney, 1999; Walther et al., 2002). If this is the case, then it might be expected that invasive species should either show a broader range of tolerances than indigenous species, or greater tolerance of warm conditions (and in the sub-Antarctic warm and dry conditions) than the latter, or a greater extent of phenotypic plasticity (for discussion of these issues for invasive species in general see Cannon, 1998; Agrawal, 2001; Stachowicz et al., 2002; Daehler, 2003; Duncan et al., 2003, and for the sub-Antarctic in particular see Kennedy, 1995; Barendse and Chown, 2000; Chown et al., 2002, Convey et al., 2006). However, these expectations have only been explicitly tested for a few species (see Stachowicz et al., 2002; Daehler, 2003; Lee et al., 2003), none of which are sub-Antarctic springtails.

Therefore, in this study we test the hypotheses that indigenous and invasive springtail species differ in their absolute thermal tolerances and extents of acclimation (a form of phenotypic plasticity, Chown and Nicolson, 2004). We do so by comparing the crystallization temperatures (= supercooling points) and upper lethal temperatures of three invasive and two indigenous springtail species from Marion Island following their acclimation to four different temperatures. Given that several studies have drawn attention to the need to explicitly ascertain the likely extent of pre-freeze mortality (e.g. Baust and Rojas, 1985; Bale, 1987, 1993), we also determine the extent to which the crystallization temperature reflects the lower lethal temperature. To do so, we apply an existing (Nedvěd et al., 1998), but poorly explored method for assessing the extent to which survival differs among different treatments by assuming that the T_c is equal to the lower lethal temperature, and then comparing this survival curve with an actual survival curve obtained under similar experimental conditions. We also examine the effects of cooling rates on springtail lower lethal temperatures because several previous studies have emphasized not only the intrinsic differences in lower lethal temperatures that might be associated with changes in cooling rate (Baust and Rojas, 1985; Worland, 2005), but also the fact that natural cooling rates differ substantially from those typically used in laboratory studies (Sinclair, 2001).

2. Materials and methods

2.1. Study site, animals and acclimation

This work was undertaken on sub-Antarctic Marion Island (46°54′S, 37°45′E), which has a cool, wet, windy climate that has shown substantial change over the last 50 years, including an increase in mean annual temperature of more than 1 °C, and a decline in precipitation of more than 500 mm per annum (Smith, 2002). The island is home to 16 springtail species, of which five are introduced (Chown et al., 2002). We investigated the five most commonly

found arthropleona species (Gabriel et al., 2001): the invasives *Pogonognathellus flavescens* Tullberg (Tomoceridae) (mean (± SE) mass 2127.53 ± 104.49 μg), *Isotomurus cf. palustris* Müller (Isotomidae) (hereafter *I. palustris*) (564.65 ± 36.58 μg) and *Ceratophysella denticulata* Bagnall (Hypogastruridae) (66.19 ± 4.99 μg); and the indigenous species *Cryptopygus antarcticus travei* Déharveng (Isotomidae) (71.48 ± 4.76 μg) and *Tullbergia bisetosa* Börner (Onychiuridae) (53.57 ± 3.73 μg). *Cryptopygus antarcticus travei* is a species in its own right, distinct from Antarctic populations of *Cryptopygus antarcticus*, though not yet formally described as such (Stevens et al., 2006a).

Specimens were collected from the field (below 25 m a.s.l.) with an aspirator and placed into 30 ml plastic vials with moist Plaster-of-Paris substrates and small amounts of detritus as a food source and for shelter. Animals were transported to the laboratory within five hours of collection. They were sorted into batches in vials, identical to those described above, for acclimation at either 0 °C (9L:15D photoperiod), 5 °C (9L:15D), 10 °C (14L:10D) or 15 °C (14L:10D) in climate chambers (accurate to ±1 °C) for no less than 7 and no more than 10 days (see Hoffmann and Watson, 1993; Terblanche et al., 2006 for rationale). Photoperiods were altered to mimic winter and summer conditions. All vials included detritus as a source of food and additional moisture. The vials occupied little space on a single incubator shelf and therefore shelf effects in the climate chambers are unlikely to have had any influence on the temperature at which the individuals were held. Moreover, the same, small areas in the climate chambers were used for each species. Comparisons among species were also performed on field fresh individuals, which were kept at ambient L:D cycles and temperatures for no longer than ten days after collection.

2.2. Crystallization temperatures and prefreeze mortality

Many studies have shown that crystallization temperatures (T_c) alone are insufficient to characterize the cold hardiness strategy of a given species because injury or death might take place either before or after freezing (reviewed in Chown and Nicolson, 2004). Pre-freeze mortality is of special concern in species that are freeze intolerant because the T_c gives no indication of the extent of pre-freeze mortality owing to chilling injury (Bale, 1993). Although it is generally accepted that for springtails the temperature at which mortality is experienced (the lower lethal temperature or LLT) is identical to the T_c (Sømme, 1982), this is not always the case, as is clear from substantial pre-freeze mortality especially in summer-acclimated individuals (e.g. van der Woude and Verhoef, 1986; Nedvěd et al., 1998). Experiments were therefore conducted to determine the extent of pre-freeze mortality, i.e. whether the instantaneous LLT differed from the T_c of each species, with the exception of *P. flavescens* (owing to constraints of the equipment used).

Springtails were acclimated at 15 °C for seven days, thus ensuring that physiological states were similar in all individuals. In the survival (LLT) experiments, batches of 10 springtails were placed in perforated Eppendorfs, which in turn were placed into 10 ml plastic tubes, each containing moist paper towel. This prevented specimens from dehydrating at temperatures above zero, whilst eliminating contact with moisture and therefore inoculative freezing at subzero temperatures. Thirteen numbered tubes, each containing 10 specimens, were submerged in a Grant LTC 12 water bath, programmed to cool from 5 to −1 °C at 0.5 °C h^{-1}, and then from −1 to −25°C at 0.1 °C min^{-1}. A thermocouple (Type T, 40 gauge) was placed inside a tube and connected to a handheld thermometer. Starting at −1 °C, one numbered tube containing 10 springtails was removed from the water bath with every 2 °C decline in temperature, as recorded by the thermocouple. After removal from the water bath, each tube was placed in an incubator set at 0 °C. Two hours after the last tube from that experiment was placed in the incubator all tubes were moved to a second incubator set to 5 °C. After 24 h at 5 °C survival was scored. Individuals that showed no signs of coordinated movement were classified as dead, while those that moved in a coordinated fashion were classified as alive. Survival scores ranging from 0% to 100% were obtained using this method. Logistic regression was used to determine the temperature at which 50% (LT50) of the sample population died for each species. LT100 was taken as the actual temperature at which the sample population suffered 100% mortality.

If pre-freeze mortality does not occur in springtails, then the T_c must represent the point of death. Based on this assumption, a survival curve can be constructed by converting the T_c of each animal in a sample population to the proportion of the population that has died. The T_c data can then be converted to survival data and compared with survival experiments undertaken under either the same conditions (as above) or under a different set of conditions. This can be done either by comparison of the logistic regression curves or using the Kaplan–Meier product limit estimation technique (Hertzberg and Leinaas, 1998). The latter procedure was followed here by examining the T_cs of a sample of springtails cooled under the same conditions using a Mettler-Toledo Differential Scanning Calorimeter (DSC) 820 (Mettler-Toledo Ltd., Leicester, UK) incorporating a mechanical intra-cooler (Lab-Plant Ltd., Huddersfield, UK) capable of cooling to −60 °C. Data were analysed using the STARe software package (Mettler-Toledo). The system was calibrated with indium (melting point 156.6 °C, enthalpy of 28.71 J g^{-1}) and regularly checked by measuring the melting point of 0.5 μl drops of HPLC grade water. Owing to instrument time constraints, repeated logistic regression curves were not constructed using the DSC approach, and only a single curve was available. Thus, the Kaplan–Meier method was used to compare this curve to the summed data from the water bath experiment described above, within each species

(excluding *P. flavescens* because of their large size). Nonetheless, the two sets of logistic curves were also inspected visually and these, together with the Kaplan–Meier statistics are reported.

2.3. The effect of cooling rate on crystallization temperature

Given that the crystallization temperature is considered an important parameter describing springtail cold hardiness (Sømme, 1982; Cannon and Block, 1988, see also Section 2.2), and because the effect of cooling rate on the T_c is controversial (Salt, 1966; Cannon, 1983; Baust and Rojas, 1985; Sinclair et al., 2003b; Worland, 2005), this aspect of cold hardiness was also investigated. For these trials, three cooling rates were employed using field fresh springtails, as well as springtails acclimated to 5 °C and to 15 °C. Crystallization temperatures were measured using a Mettler-Toledo Differential Scanning Calorimeter as above. Springtails were hermetically sealed in 40 µl aluminium pans and cooled at one of three cooling rates. The first cooling rate protocol (labelled fast) was the standard 1 °C min^{-1} cooling rate (Chown and Nicolson, 2004), whereas the second protocol (labelled medium) employed a slower cooling rate of 0.1 °C min^{-1}. For both of these protocols, samples were cooled from 5 to −30 °C at their respective cooling rates, following a 5 min equilibration period at 5 °C. In the third protocol (labelled slow), however, a slower cooling approach, similar to that used in assessments of rapid responses of the T_c to changing temperature (Worland and Convey, 2001), was used. Here, cooling commenced at 5 °C and the temperature was lowered to −1 °C at 0.5 °C h^{-1}. From −1 to −30 °C a cooling rate of 0.1 °C min^{-1} was employed. Because the T_c is assumed to be equivalent to the LLT in springtails, this protocol also enabled us to determine the extent to which springtails rapidly cold harden (see Chown and Nicolson, 2004). The lowest temperature recorded prior to a freezing exotherm was taken as the T_c of that individual in all instances. Sample sizes exceeded 20 in all instances. Experiments performed in the DSC precluded investigations of *P. flavescens* due to the large size of this species. Crystallization temperatures following different cooling rates were compared within species using generalized linear models (assuming a normal distribution of errors and using identity link functions).

2.4. Responses of crystallization temperatures to acclimation

The responses of T_c to acclimation were investigated in all of the species with the exception of *Ceratophysella denticulata* for which only two acclimation treatments were available owing to incubator failure. Because the differential scanning calorimeter was available for a limited period only, more conventional techniques for estimating T_c were used for these experiments. The T_c of at least 20 individual specimens per species and treatment (0, 5, 15 and 20 °C) were determined with Type T copper–constantan thermocouples (40-gauge) connected to a Campbell CR10 datalogger. Specimens ($n = 10$) were placed in vacutest vials (BD Vacutainer Systems, UK) and submerged in a Grant LTC 12 water bath at 0 °C for those animals acclimated at 0 °C, and 5 °C for field fresh animals and those acclimated at 5, 15 and 20 °C, respectively. Water bath temperature was lowered by 0.1 °C min^{-1} after a 15 min equilibration period at 0 or 5 °C. The lowest temperature recorded prior to the onset of a freezing exotherm was taken as the T_c for that individual. These experiments were also conducted on field fresh individuals for each of the species investigated to assess baseline (field) supercooling ability. Because the T_c values were not normally distributed (although they were not bimodal, see, e.g. Fig. 1) the effects of species, acclimation treatment, and their interaction were investigated using a generalized linear model assuming a normal distribution of errors and using a log link function. This model excluded *Ceratophysella denticulata*. Where the effects of acclimation were considered within species, separate models were built in each case. In addition, T_cs from field fresh individuals of each species were compared in a separate assessment using a generalized linear model assuming a normal distribution of errors and using an identity link function.

2.5. Upper lethal temperatures

To test the responses of upper lethal temperatures to different acclimation temperatures (0, 5, 15 and, in two species, 20 °C), mortality assays were conducted on springtails exposed to different test temperatures. The 20 °C treatments were not assessed for *Cryptopygus antarcticus*, *Tullbergia bisetosa* and *Ceratophysella denticulata* because mortality was typically high in these treatments, often within less than two days in the indigenous species. For the ULT experiments, batches of ten individuals were placed into plastic tubes, containing moist filter paper to avoid the confounding effects of desiccation (see Hoffmann et al., 2003), which were submerged into Grant LTD20 or LTC12 water baths at the set temperatures for one hour. Tubes were removed from the water bath, supplied with new moist filter paper, and transferred to an incubator (at the original acclimation temperature, except for field fresh batches that were transferred to a 10 °C incubator). Survival was scored after 24 h. Individuals that showed coordinated movement were scored as alive, whereas those that did not were scored as dead. Water bath temperature was increased by 1 °C and the experiment repeated with new individuals until the temperature range encompassed 5–95% survival. The procedure was replicated at least four times, but generally five times at each experimental temperature for the field fresh and each of the acclimation temperatures for each species. Logistic regression was used to determine the temperature at which 50% (LT50) of the sample population died for each species and acclimation temperature (0, 5, 15 and 20 °C). Only data sets for which

significant Wald's statistics were obtained were used in comparison within treatments. The effect of species, acclimation, and the species by acclimation interaction on LT50 was investigated using a general linear model with data from the 0, 5 and 15 °C acclimation treatments only. Two further analyses of variance were used to investigate the effects of the 20 °C treatment, relative to the others, in *P. flavescens* and *I. palustris*.

3. Results

In field fresh individuals, *I. palustris* had a significantly higher mean T_c than any other species in this study, whereas the indigenous *Tullbergia bisetosa* displayed the lowest mean T_c (Table 1). The logistic regressions of both the survival and T_c experiments, and the Kaplan–Meier statistics used to compare LLT and LLT calculated from T_c (Fig. 2) indicated an absence of pre-freeze mortality in all of the species. Cooling rate typically also had no significant effect on crystallization temperature (Table 2). Where effects were found these tended to be idiosyncratic. For example, in field fresh *Ceratophysella denticulata* T_c increased with a more rapid cooling rate, in field fresh *Cryptopygus antarcticus* T_c declined with more rapid cooling, and in *I. palustris* acclimated to 15 °C a unimodal response was found, with the lowest T_c at the intermediate cooling rate.

In *I. palustris* and *Tullbergia bisetosa* T_c declined with declining acclimation temperature, whereas the opposite was found for *P. flavescens* (Table 3 and Fig. 3). The

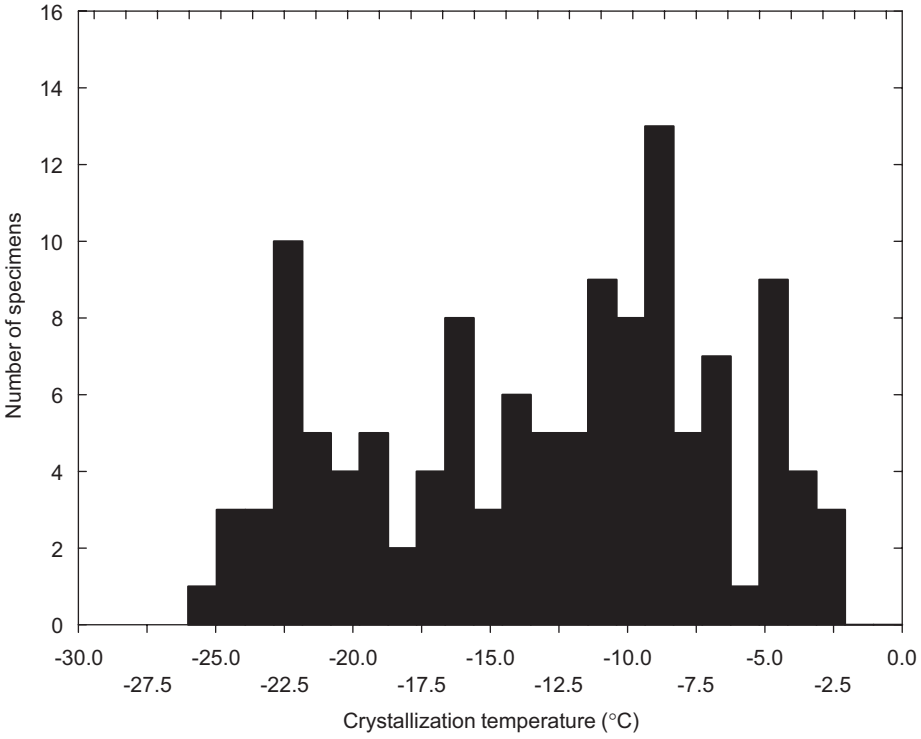

Fig. 1. Frequency distribution of crystallization temperatures for *Cryptopygus antarcticus travei* individuals acclimated to 5 °C indicating lack of bimodality that is typical of this species in other, more southerly, locations.

Table 1
Summary statistics for crystallization temperatures (°C) of field-fresh springtails

	n	Mean ± S.E.	Median	Minimum	Maximum
Invasive					
Pogonognathellus flavescens	31	-12.0 ± 1.00^{ab}	−11.2	−21.5	−3.2
Isotomurus palustris	24	-8.2 ± 1.24^{a}	−5.2	−24.4	−3.5
Ceratophysella denticulata	46	-14.3 ± 0.93^{b}	−13.9	−22.9	−3.1
Indigenous					
Cryptopygus antarcticus	25	-13.5 ± 0.71^{b}	−13.0	−22.6	−8.8
Tullbergia bisetosa	20	-19.4 ± 0.90^{c}	−20.9	−25.2	−12.6
$\chi^2_{(4)} = 43.93$	$p < 0.0001$				

Different letters for crystallization temperatures denote significantly different means based on the 95% confidence intervals of the weighted marginal means obtained from fitting a generalized model with normal errors and an identity link function to the data.

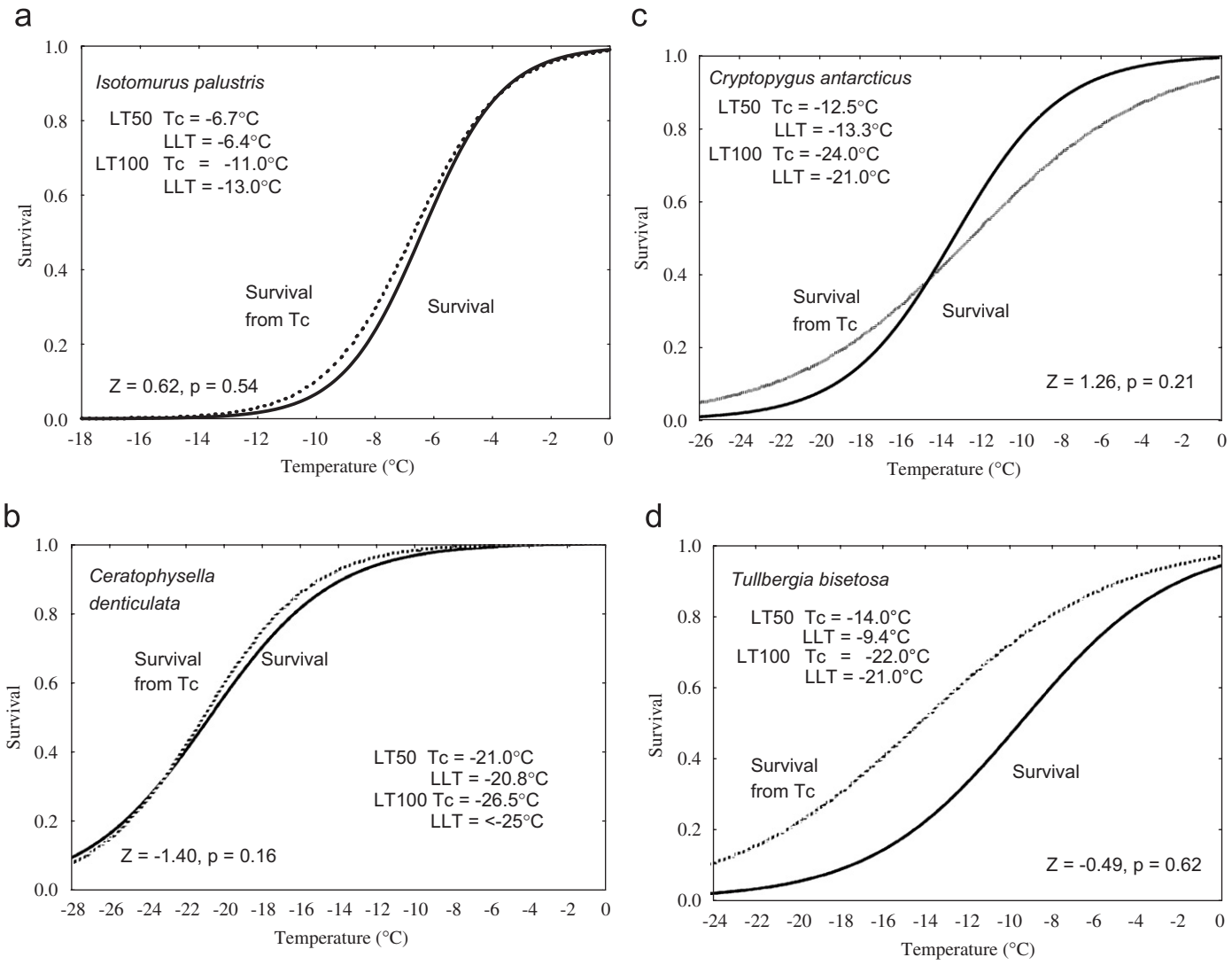

Fig. 2. Survival curves of Collembola acclimated at 15 °C calculated from actual survival and assuming that the crystallization temperature is equivalent to the lower lethal temperature (survival from T_c). Curves were constructed using logistic regression statistics, but comparisons between curves were made using the Kaplan–Meier statistic (Z), which is reported here together with its significance. The temperatures at which 50% (LT50) and 100% of the sample (LT100) died, for both the survival (LLT) and crystallization temperature (T_c) trials, are also provided: (a) *Isotomurus palustris*; (b) *Ceratophysella denticulata*; (c) *Cryptopygus antarcticus travei*; (d) *Tullbergia bisetosa.*

magnitude of these changes was not large, ranging from 4 to 6 °C. Crystallization temperatures were unaffected by acclimation treatment in *Ceratophysella denticulata* (separate model, $\chi^2 = 2.54$, $p > 0.05$) and *Cryptopygus antarcticus* (Fig. 3). Although interspecific differences in T_cs were not always apparent in this experiment at the higher acclimation temperatures, at the lower acclimation temperatures significant differences in T_c were clear. With the exception of *Ceratophysella denticulata*, the invasive species had higher T_cs than the indigenous ones.

By contrast, the invasive alien springtail species had higher upper lethal temperatures than the indigenous species (Table 4 and Fig. 4), and the alien *Ceratophysella denticulata* had a significantly higher ULT50 than all of the other species. The responses to acclimation also differed between the species, so accounting for the significant interaction term in the model (Table 4). Typically, the invasive alien species showed a substantial increase in ULT50 with increasing acclimation temperature (see also Fig. 5), whereas in the indigenous *Tullbergia bisetosa* the effect was smaller, and in *Cryptopygus antarcticus* precisely the opposite effect was found (Fig. 4).

4. Discussion

Twenty years ago, two papers (Baust and Rojas, 1985; Bale, 1987) led to dramatic changes in the field of insect cold hardiness. In particular, these papers emphasized that cooling rates might substantially alter estimates of lower lethal temperatures, that pre-freeze mortality is likely to be important in many freezing intolerant species, and that cold hardiness strategies cannot be fully comprehended

Table 2
Summary statistics of the effect of cooling rate (fast medium and slow rates) on crystallization temperature (°C ± SE) for unacclimated (field fresh (FF)) springtails, and those acclimated at 5 and 15 °C

Species	Rate	*n*	Mean ± S.E.	
Invasive				
Isotomurus palustris				
FF	Fast	28	−9.1 ± 0.71	$\chi^2_{(2)} = 2.88$
	Medium	32	−7.9 ± 0.41	$p > 0.05$
	Slow	20	−7.8 ± 0.83	
5 °C	Fast	43	−5.9 ± 0.55	$\chi^2_{(2)} = 0.77$
	Medium	40	−5.3 ± 0.68	$p > 0.05$
	Slow	25	−6.1 ± 0.72	
15 °C	Fast	46	−6.3 ± 0.53	$\chi^2_{(2)} = 14.29$
	Medium	42	−4.4 ± 0.32	$p < 0.05$
	Slow	20	−7.5 ± 0.92	
Ceratophysella denticulata				
FF	Fast	40	−10.8 ± 0.95	$\chi^2_{(2)} = 12.99$
	Medium	46	−14.3 ± 0.93	$p < 0.05$
	Slow	24	−16.1 ± 1.10	
5 °C	Fast	49	−17.0 ± 0.96	$\chi^2_{(2)} = 2.83$
	Medium	58	−17.7 ± 0.78	$p > 0.05$
	Slow	31	−19.3 ± 0.95	
15 °C	Fast	65	−20.7 ± 0.53	$\chi^2_{(2)} = 3.78$
	Medium	58	−19.4 ± 0.72	$p > 0.05$
	Slow	40	−21.2 ± 0.71	
Indigenous				
Cryptopygus antarcticus				
FF	Fast	86	−15.7 ± 0.47	$\chi^2_{(2)} = 10.40$
	Medium	53	−13.0 ± 0.75	$p < 0.05$
	Slow	22	−13.0 ± 1.56	
5 °C	Fast	44	−13.3 ± 0.87	$\chi^2_{(2)} = 0.12$
	Medium	50	−12.9 ± 0.90	$p > 0.05$
	Slow	29	−13.1 ± 1.30	
15 °C	Fast	41	−11.6 ± 1.07	$\chi^2_{(2)} = 1.30$
	Medium	43	−10.8 ± 1.10	$p > 0.05$
	Slow	24	−12.9 ± 1.42	
Tullbergia bisetosa				
FF	Fast	37	−14.4 ± 0.78	$\chi^2_{(2)} = 2.64$
	Medium	41	−12.4 ± 0.88	$p > 0.05$
	Slow	25	−13.5 ± 1.10	
5 °C	Fast	48	−14.8 ± 0.65	$\chi^2_{(2)} = 5.72$
	Medium	52	−14.0 ± 0.76	$p > 0.05$
	Slow	30	−16.9 ± 1.13	
15 °C	Fast	45	−15.2 ± 0.87	$\chi^2_{(2)} = 0.93$
	Medium	50	−15.5 ± 0.87	$p > 0.05$
	Slow	29	−14.1 ± 1.20	

Generalized linear models assuming a normal distribution of errors and using identity link functions were fitted to the data.

without an understanding of their environmental context. These caveats have borne substantial fruit. It is now widely appreciated that a variety of responses to low temperature exists in arthropods (Bale, 2002; Sinclair et al., 2003b; Sinclair and Chown, 2005a), and that these responses can vary over surprisingly short time-scales (Kelty and Lee, 2001; Worland and Convey, 2001). Similarly, the significance of determining the likelihood and extent of chilling injury during investigations of cold hardiness is now broadly acknowledged (see Chown and Nicolson, 2004 for review).

In this study, the likelihood of chilling injury over the short term, i.e. instantaneous pre-freeze mortality, was investigated explicitly using a technique that directly compares the actual survival curve with one estimated by assuming that individuals die at, but not before, the point at which they freeze (T_c). In the absence of pre-freeze mortality the two curves should be identical, whereas if

Table 3
Outcome of a generalized linear model (assuming normal errors and using an identity link function) investigating the effect of species and acclimation treatment, and their interaction on the crystallization temperature (= lower lethal temperature in these species)

Effect	χ^2	df	p
Species	150.91	3	0.00001
Acclimation	6.3	2	0.043
Species × Acclimation	15.3	6	0.018
Deviance/df	5515/234 = 23.6		

This model does not include *Ceratophysella denticulata*, for which data at 0 and 20 °C were not available.

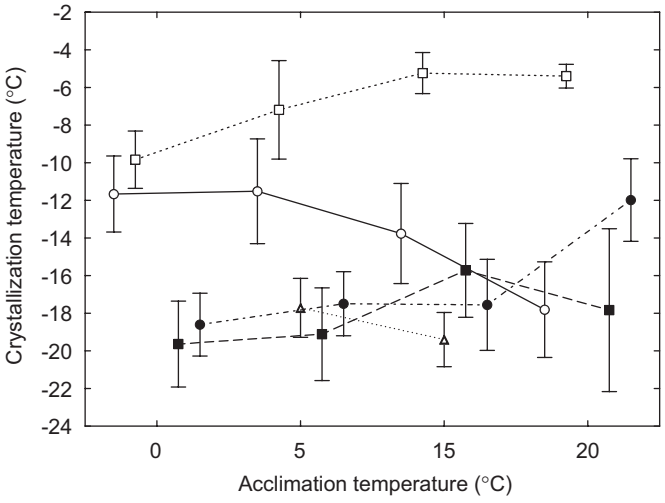

Fig. 3. Interspecific differences in the effect of acclimation on the crystallization temperature (mean ± 95% confidence intervals) for all five springtail species examined here: Invasive: *P. flavescens* (○), *I. palustris* (□), *Ceratophysella denticulata* (△); Indigenous: *Cryptopygus antarcticus travei* (■), *Tullbergia bisetosa* (●). Note that means are shifted along the *x*-axis for each temperature so that the confidence intervals can be distinguished for each species and treatment.

Table 4
Outcome of a general linear model investigating the effect of species and acclimation treatment, and their interaction, on the upper temperature at which 50% of the sample of springtails survived

Effect	Sums of squares	df	F	p
Intercept	81458.8	1	598471	0.00001
Species	248.2	4	455.9	0.00001
Acclimation	4.2	2	15.5	0.00001
Species × Acclimation	32.4	8	29.8	0.00001
Error		59		

Note the significant interaction term.

chilling injury occurs the actual survival curve should have a form very different to that of the survival curve estimated from the T_c. In the species examined here the curves were not statistically distinguishable based on the analytical techniques we used. Therefore, it is clear that, over the short term, the crystallization temperature (or supercooling

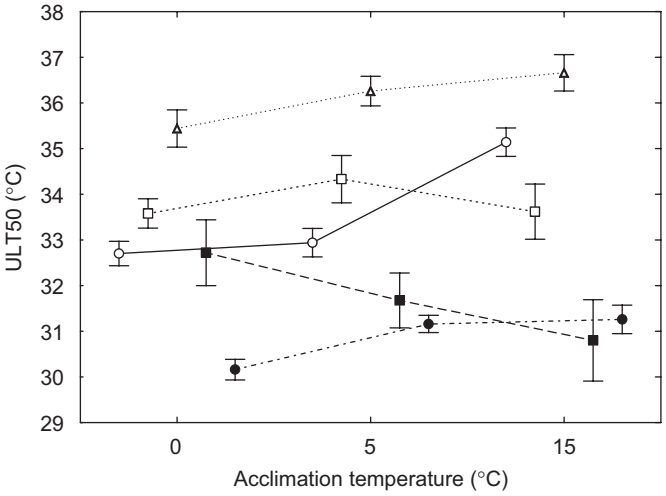

Fig. 4. Interspecific differences in the effect of acclimation on the upper temperature at which 50% of the sample of animals survived (ULT50) (mean ± 95% confidence intervals) for all five springtail species examined here: Invasive: *P. flavescens* (○), *I. palustris* (□), *Ceratophysella denticulata* (△); Indigenous: *Cryptopygus antarcticus travei* (■), *Tullbergia bisetosa* (●). Note that means are shifted along the *x*-axis for each temperature so that the confidence intervals can be distinguished for each species and treatment.

point) represents the lower lethal temperature. The absence of pre-freeze mortality in these four springtail species bears out previous findings that, in general, the T_c represents the lower lethal temperature in springtails (Sømme, 1982; Cannon and Block, 1988; Sinclair and Sjursen, 2001; Sinclair et al., 2006). Moreover, given that the trials were undertaken following acclimation of individuals to 15 °C (i.e. summer-like conditions) the results contrast strongly with those for other species where summer-acclimated individuals show substantial chilling injury (e.g. Nedvěd et al., 1998). The reasons for these differences is not clear, but the limited response of T_c to acclimation, at least over a 0–15 °C acclimation temperature range in the species examined here, suggests that the species tend to retain their cold hardiness over the range of temperatures typically encountered in the field (see below and Deere et al., 2006 for microclimate data). Small changes in cold hardiness in the face of different acclimation temperatures are typical of some (e.g. Slabber and Chown, 2005; Deere et al., 2006), but not all (Klok and Chown, 1998; Slabber and Chown, 2004) arthropods on Marion Island.

The absence of short-term pre-freeze mortality does not mean that over longer periods chilling injury or the risk of death owing to freezing might not be significant. Indeed, given that many studies have documented an increase in chilling injury with time at low temperature (see Bale 1987, 2002), and given the metastable nature of the supercooled state (Sømme, 1999; Ramløv, 2000), mortality at subzero temperatures higher than the T_c might be expected over longer experimental durations than those used here. Whilst we did not examine the extent to which such mortality might have been realized, the logistic-regression techniques could readily be used to do so (see also Nedvěd et al.,

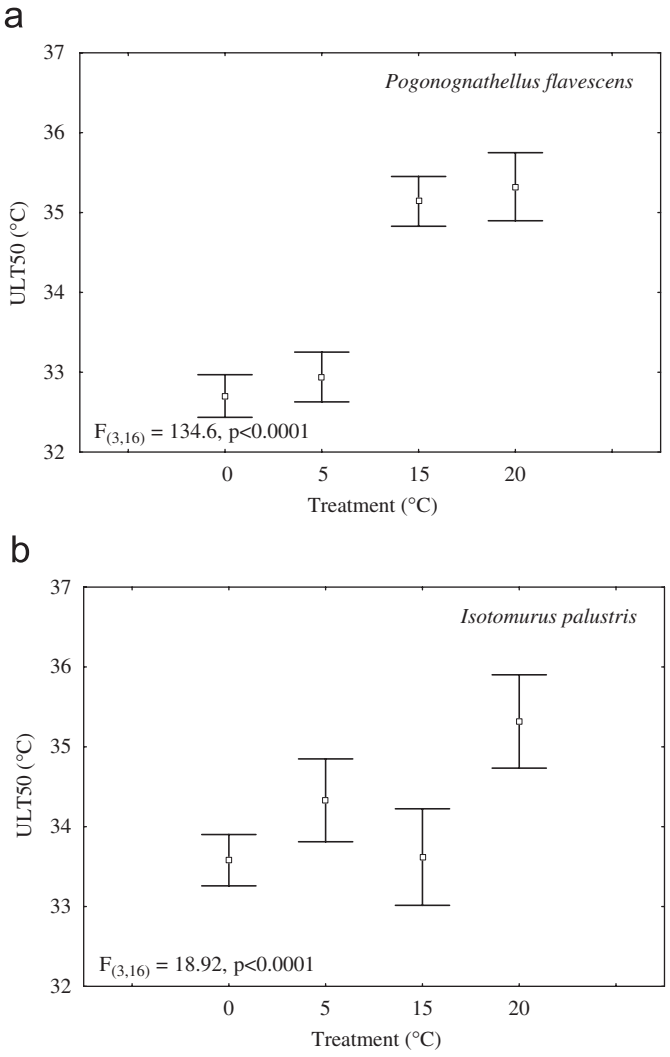

Fig. 5. The effect of acclimation on the upper temperature at which 50% of the sample of animals survived (ULT 50) (mean ± 95% confidence intervals) following acclimation at 0, 5, 15 and 20 °C in (a) *Pogonognathellus flavescens* and (b) *Isotomurus palustris*.

1998). Indeed, comparison of survival curves obtained from holding animals at different times at a given low temperature, with survival curves derived from the T_c might provide substantial insight into the time course of chilling injury relative to freezing injury. Records of body temperatures during long-term survival experiments could provide similar comparative data on the significance of chilling vs. freezing injury (see Bale, 1987), which would be amenable to such analysis too. Nonetheless, given the short duration of low sub-zero temperatures at Marion Island (Chown and Crafford, 1992; Sinclair and Chown, 2005b) we do not think prolonged low temperature exposure to be especially likely in this environment.

In the species studied here, crystallization temperature, and therefore lower lethal temperature, varied little with cooling rate (which varied from 1 °C min^{-1} to less than 0.1 °C min^{-1}). Where differences in T_c among cooling treatments were significant, the differences themselves were typically small. The exception was *Ceratophysella denticulata*, but for field fresh individuals the data may have been biased by differences in moulting state of the animals (see Worland et al., 2006). These results differ from those found for other springtail species (Cannon, 1983; Worland, 2005) where rate of cooling has a marked effect on T_c. Moreover, they also differ from the only other study that has adopted the slow cooling approach (i.e. biphasic cooling) used here. Worland and Convey (2001) found that in *Cryptopygus antarcticus* from the Antarctic Peninsula very slow cooling (0.5 °C h^{-1} from +5 to −5 °C) followed by a standard cooling rate of 1 °C min^{-1} substantially lowered T_c by comparison with individuals cooled at 1 °C min^{-1}. Whether crystallization temperature would have been more substantially affected by either much faster or much slower cooling rates is difficult to determine. However, given that Worland (2005) found a steep unimodal relationship between cooling rate and T_c in *Tullbergia antarctica* from the Kerguelen Islands, and that Sinclair (2001) has emphasized the importance of considering very slow cooling rates, this possibility deserves further investigation.

Having established that T_cs and lower lethal temperatures are equivalent, at least over the short-term, and that they are insensitive to the range of experimental conditions adopted here, the question remains as to whether these T_cs bare any relation to the environmental temperatures likely to be encountered by the species on Marion Island. In this context, much of the literature has rightly been concerned with discussion of long-term survival of low temperatures (Bale, 1987; Leather et al., 1993; Sømme, 1999). However, it is also clear that, as far as selection is concerned, short-term extreme events are of considerable significance (Gaines and Denny, 1993; Chown and Terblanche, 2007). Indeed, it is such short-term extreme events that are likely to determine the composition of the population in terms of its absolute physiological tolerances over the longer term (see also Sinclair, 2001). Therefore, understanding the environmental relevance of a particular lower (or upper) lethal temperature requires understanding not only of long-term mean conditions, but, perhaps more significantly, also of short-term extremes. To this end, we examined the monthly absolute minimum temperatures recorded (using Thermochron ibuttons, Dallas Semi-Conductors Model DS1921) just below the soil surface at 200 m intervals from sea-level to 800 m on Marion Island over the period May 2002–April 2004 (see Slabber and Chown, 2005; Deere et al., 2006 for additional detail on the microclimate data). It is clear that the mean (or median, Table 1 and Fig. 3) T_cs are unlikely to be exceeded in most environments (Table 5) in the indigenous species, although this might be the case for the invasive species *I. palustris* and *P. flavescens* either at very high altitudes, where low temperature reach their most extreme values, or altitudes where snow cover is typically absent but temperatures may still be low (200 m).

That the T_cs of the indigenous species reflect the microhabitat temperatures they encounter is also indicated by comparisons of data from these species with those from

Table 5
Microhabitat temperatures (°C) of nine terrestrial sites (0–800 m a.s.l.) for two years at Marion Island

	Year	Mean	Abs. min	Abs. max	Mean min	Mean max	Range
0 m	2002	5.3	0.5	22.5	3.8	7.1	22.0
	2003	7.0	1.0	22.0	5.1	8.6	21.0
100 m	2002	5.2	0.0	16.0	3.1	7.7	16.0
	2003	5.7	0.5	17.5	3.7	8.1	17.0
200 m	2002	4.4	−5.0	18.5	2.2	7.0	23.5
	2003	5.2	−6.0	19.0	3.5	6.6	25.0
300 m	2002	4.1	0.0	15.0	2.4	6.1	15.0
	2003	4.2	−1.0	15.5	2.4	6.3	16.5
400 m	2002	2.8	−1.0	13.0	1.8	5.7	14.0
	2003	3.1	−0.5	12.5	1.9	6.0	13.0
500 m	2002	3.5	0.0	24.0	1.8	6.1	24.0
	2003	3.6	−6.5	30.0	1.6	6.4	36.5
600 m	2002	2.5	0.0	11.5	1.8	4.1	11.5
	2003	2.7	−0.5	13.0	1.9	5.0	13.5
700 m	2002	2.5	−7.0	17.5	0.5	4.8	24.5
	2003	3.0	−1.5	22.5	1.4	5.2	24.0
800 m	2002	1.5	−12.0	23.0	−0.3	3.6	35.0
	2003	1.8	−4.5	19.5	0.3	3.6	24.0

Mean, mean minimum (Mean min) and mean maximum (Mean max), absolute minimum (Abs min) and absolute maximum (Abs max) temperatures, and temperature range are given for each site for each year. Terrestrial data were collected using Thermochron ibutton loggers (Dallas Semiconductors, Model DS 1921) placed just below the soil surface (for additional information see Deere et al., 2006).

species found elsewhere in the broader Antarctic region and with data from other populations of the broadly distributed, Antarctic species flock *Cryptopygus antarcticus* (see Stevens et al., 2006a). Populations of the latter species have been extensively investigated both at Signy Island and Rothera Point (a more southerly, Antarctic Peninsula location). At these locations, T_c distributions in *Cryptopygus antarcticus* are strongly bimodal, unlike the case here (compare Fig. 1 with the figures for this species provided by Block, 1982b; Sømme and Block, 1982), and the T_cs in the low group of the bimodal distribution tend to be lower by several degrees than those found here. Nonetheless, in some instances little difference exists between the T_c values found for *Cryptopygus antarcticus travei* from Marion Island and those found for populations from elsewhere in the Antarctic (e.g. Cannon, 1983; Worland and Convey, 2001). In these cases the Antarctic populations were either examined in late summer or had been subject to prolonged acclimation at a relatively high temperature presumably resulting in a shift in T_c away from the lower values more typical of winter populations. In comparison with other Antarctic species it is clear that the mean T_c of the indigenous species examined here are relatively high. For example, *Cryptopygus sverdrupi* from the continental Antarctic has a T_c of c. −35 °C (Sømme, 1986b), whilst those of *Gomphiocephalus hodgsoni* from the Ross Sea region reach similarly low values (Sinclair and Sjursen, 2001). In *Isotoma klovstadi* (now *Desoria klovstadi*—see Stevens et al., 2006b) another species from the Ross Sea region, summer low group T_cs may be as low as −35 °C (Sinclair et al., 2006), and it is in this species that Pryor (1962) reported overwinter survival of temperatures lower than −50 °C. The mean T_c of *Cryptopygus cisantarcticus* (c. −28 °C), a species also found in the Ross Sea region (Sinclair et al., 2006), is several degrees lower than the mean T_c values found for *Cryptopygus antarcticus* and *Tullbergia bisetosa*. By contrast, *Tullbergia antarctica* a species characteristic of the warmer, sub-Antarctic Kerguelen Island has a T_c of c. −9 to −12 °C, much closer to those recorded in the indigenous species in this study.

Particularly at the lower acclimation temperatures the invasive species, with the exception of *Ceratophysella denticulata*, had T_cs much higher than those found in the indigenous species. The values found for *P. flavescens* are similar to the lower lethal temperatures found in a related European species, *Tomocerus minor* (van der Woude and Verhoef, 1988). Differences in the T_cs of the indigenous and invasive species probably account at least partly for the restriction of the invasive species to lowland habitats, but the wider occurrence of the indigenous species especially at high elevations on the island (Barendse and Chown, 2001; Gabriel et al., 2001; McGeoch et al., 2006). These differences in distribution are also likely a consequence of difference in desiccation tolerance responses to temperature in these species (see Gabriel et al., 2001; Slabber et al., submitted). However, repeated exposures to subzero temperatures might also have differential effects on the indigenous vs. the invasive species, but the effects of

repeated sublethal exposures on these (or other) springtails has not been extensively investigated (see Nedvěd et al., 1998; Renault et al., 2004 for a similar approach, but examining the beneficial effects of high temperature respites).

By the same rationale as that followed above, it appears that the upper lethal temperatures are unlikely to be exceeded in the invasive species, but that this might occasionally occur in the indigenous species. In the latter, ULT50s are in the vicinity of 30 °C, and, at least at some sites, in some years, microhabitat temperatures can reach this value (Table 5, see also Chown and Crafford, 1992). In this regard it is noteworthy that whilst acclimation to higher temperatures tended to improve the ULT50 in the invasive species, in the indigenous species increasing acclimation temperature either resulted in a decline in (*Cryptopygus antarcticus*) or little change to the ULT50 (*Tullbergia bisetosa*).

Returning to the question of whether the indigenous and invasive species show systematic differences in tolerances and the response of upper and lower lethal limits to acclimation, it is clear that this is largely the case. The indigenous species tend to be more tolerant of low temperatures and less tolerant of high temperatures, although the invasive *Ceratophysella denticulata* is an exception. In the case of acclimation effects, the response of T_c tended not to differ among the two groups of species, although the very strong negative response of T_c to higher acclimation temperatures in *P. flavescens* means that at the lower acclimation temperatures the T_cs of the indigenous and invasive species differ, whereas such a distinction is less clear at the higher temperatures. By contrast, the ULT50 showed very different responses among the indigenous and invasive species, with elevated acclimation temperatures tending to increase ULT50 in the invasive species, whilst having either the converse effect, or no pronounced effect on the indigenous species. A similar response of desiccation resistance to thermal acclimation was found in these species, with resistance improving following acclimation to high temperature in the invasive species, but declining in the indigenous species (Slabber et al., submitted). Thus, it seems likely that with ongoing climate change at the island, which has already warmed by more than 1 °C and lost more than 500 mm mean annual precipitation over the past 50 years (Smith, 2002), the invasive species will be favoured over the indigenous ones. This prediction has been borne out by manipulative field experiments which have demonstrated that with warming and drying the indigenous species decline substantially in abundance, whereas the invasive species are little affected (McGeoch et al., 2006). Thus, climate change is likely to favour some invasive over indigenous springtails at Marion Island, a finding partially in keeping with the generalizations made in the literature on the likely interactions between biological invasions and climate warming (Dukes and Mooney, 1999; Walther et al., 2002; Frenot et al., 2005).

Acknowledgments

We thank Erika Nortje for assistance in the laboratory, and John Terblanche and two anonymous referees for comments on the manuscript. This work was supported by the DST-NRF Centre of Excellence for Invasion Biology, a South African National Research Foundation/Norwegian Research Council Science Liaison Grant, and by the SU/DEAT/USAID Capacity Building Programme for Climate Change Research. It is a contribution to the SCAR EBA programme.

References

Agrawal, A.A., 2001. Phenotypic plasticity in the interactions and evolution of species. Science 294, 321–326.

Bale, J.S., 1987. Insect cold hardiness: freezing and supercooling—an ecophysiological perspective. Journal of Insect Physiology 33, 899–908.

Bale, J.S., 1993. Classes of insect cold hardiness. Functional Ecology 7, 751–753.

Bale, J.S., 2002. Insects and low temperatures: from molecular biology to distributions and abundance. Philosophical Transactions of the Royal Society of London B 357, 849–861.

Barendse, J., Chown, S.L., 2000. The biology of *Bothrometopus elongatus* (Coleoptera, Curculionidae) in a mid-altitude fellfield on sub-Antarctic Marion Island. Polar Biology 23, 346–351.

Barendse, J., Chown, S.L., 2001. Abundance and seasonality of mid-altitude fellfield arthropods from Marion Island. Polar Biology 24, 73–82.

Baust, J.G., Rojas, R.R., 1985. Insect cold hardiness: facts and fancy. Journal of Insect Physiology 31, 755–759.

Bergstrom, D., Chown, S.L., 1999. Life at the front: history, ecology and change on southern ocean islands. Trends in Ecology and Evolution 14, 472–477.

Block, W., 1982a. The Signy Island terrestrial reference sites: XIV. Population studies on the Collembola. British Antarctic Survey Bulletin 55, 33–49.

Block, W., 1982b. Supercooling points of insects and mites on the Antarctic Peninsula. Ecological Entomology 7, 1–8.

Block, W., 1984. Terrestrial microbiology, invertebrates and ecosystems. In: Laws, R.M. (Ed.), Antarctic Ecology, vol. 1. Academic Press, London, pp. 163–236.

Cannon, R.J.C., 1983. Experimental studies on supercooling in two Antarctic micro-arthropods. Journal of Insect Physiology 29, 617–624.

Cannon, R.J.C., 1986. Diet and acclimation effects on the cold tolerance and survival of an Antarctic springtail. British Antarctic Survey Bulletin 71, 19–30.

Cannon, R.J.C., 1998. The implications of predicted climate change for insect pests in the UK, with emphasis on non-indigenous species. Global Change Biology 4, 785–796.

Cannon, R.J.C., Block, W., 1988. Cold tolerance of microarthropods. Biological Reviews 63, 23–77.

Chown, S.L., Convey, P., 2007. Spatial and temporal variability across life's hierarchies in the terrestrial Antarctic. Philosophical Transactions of the Royal Society of London B, in press.

Chown, S.L., Crafford, J.E., 1992. Microhabitat temperatures at Marion Island (46°54′S 37°45′E). South African Journal of Antarctic Research 22, 51–58.

Chown, S.L., Nicolson, S.W., 2004. Insect Physiological Ecology. Mechanisms and Patterns. Oxford University Press, Oxford.

Chown, S.L., Terblanche, J.S., 2007. Physiological diversity in insects: ecological and evolutionary contexts. Advances in Insect Physiology, in press.

Chown, S.L., Gremmen, N.J.M., Gaston, K.J., 1998. Ecological biogeography of southern ocean islands: species–area relationships,

human impacts, and conservation. American Naturalist 152, 562–575.
Chown, S.L., McGeoch, M.A., Marshall, D.J., 2002. Diversity and conservation of invertebrates on the sub-Antarctic Prince Edward Islands. African Entomology 10, 67–82.
Convey, P., McInnes, S.J., 2005. Exceptional tardigrade-dominated ecosystems in Ellsworth Land, Antarctica. Ecology 86, 519–527.
Convey, P., Greenslade, P., Arnold, R.J., Block, W., 1999. Collembola of sub-Antarctic South Georgia. Polar Biology 22, 1–6.
Convey, P., Greenslade, P., Pugh, P.J.A., 2000. The terrestrial microarthropod fauna of the South Sandwich Islands. Journal of Natural History 34, 597–609.
Convey, P., Chown, S.L., Wasley, J., Bergstrom, D.M., 2006. Life history traits. In: Huiskes, A.H.L., Bergstrom, D., Convey, P. (Eds.), Trends in Antarctic Terrestrial and Limnetic Ecosystems. Springer, Berlin.
Daehler, C.C., 2003. Performance comparisons of co-occurring native and alien invasive plants: implications for conservation and restoration. Annual Review of Ecology, Evolution and Systematics 34, 183–211.
Deere, J.A., Sinclair, B.J., Marshall, D.J., Chown, S.L., 2006. Phenotypic plasticity of thermal tolerances in five oribatid mite species from sub-Antarctic Marion Island. Journal of Insect Physiology 52, 693–700.
Dukes, J.S., Mooney, H.A., 1999. Does global change increase the success of biological invaders? Trends in Ecology and Evolution 14, 135–139.
Duncan, R.P., Blackburn, T.M., Sol, D., 2003. The ecology of bird introductions. Annual Review of Ecology, Evolution and Systematics 34, 71–98.
Frenot, Y., Chown, S.L., Whinam, J., Selkirk, P.M., Convey, P., Skotnicki, M., Bergstrom, D.M., 2005. Biological invasions in the Antarctic: extent, impacts and implications. Biological Reviews 80, 45–72.
Gabriel, A.G.A., Chown, S.L., Barendse, J., Marshall, D.J., Mercer, R.D., Pugh, P.J.A., Smith, V.R., 2001. Biological invasions on Southern Ocean islands: the Collembola of Marion Island as a test of generalities. Ecography 24, 421–430.
Gaines, S.D., Denny, M.W., 1993. The largest, smallest, highest, lowest, longest, and shortest: extremes in ecology. Ecology 74, 1677–1692.
Greenslade, P., 1990. Notes on the biogeography of the free-living terrestrial invertebrate fauna of Macquarie Island with an annotated checklist. Papers and Proceedings of the Royal Society of Tasmania 124, 35–50.
Harrisson, P.M., Rothery, P., Block, W., 1991. Drying processes in the Antarctic Collembolan *Cryptopygus antarcticus* (Willem). Journal of Insect Physiology 37, 883–890.
Hayward, S.A.L., Bale, J.S., Worland, M.R., Convey, P., 2001. Influence of temperature on the hygropreference of the Collembolan, *Cryptopygus antarcticus*, and the mite, *Alaskozetes antarcticus* from the maritime Antarctic. Journal of Insect Physiology 47, 11–18.
Hertzberg, K., Leinaas, H.P., 1998. Drought stress as a mortality factor in two pairs of sympatric species of Collembola at Spitsbergen, Svalbard. Polar Biology 19, 302–306.
Hoffmann, A.A., Watson, M., 1993. Geographical variation in the acclimation responses of *Drosophila* to temperature extremes. American Naturalist 142, S93–S113.
Hoffmann, A.A., Sørensen, J.G., Loeschcke, V., 2003. Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. Journal of Thermal Biology 28, 175–216.
Hugo, E.A., McGeoch, M.A., Marshall, D.J., 2004. Fine scale variation in microarthropod communities inhabiting the keystone species *Azorella selago* on Marion Island. Polar Biology 27, 466–473.
Kelty, J.D., Lee, R.E., 2001. Rapid cold-hardening of *Drosophila melanogaster* (Diptera: Drosophilidae) during ecologically based thermoperiodic cycles. Journal of Experimental Biology 204, 1659–1666.
Kennedy, A.D., 1995. Antarctic terrestrial ecosystem response to global environmental change. Annual Review of Ecology and Systematics 26, 683–704.
Klok, C.J., Chown, S.L., 1998. Interactions between desiccation resistance, host–plant contact and the thermal biology of a leaf-dwelling sub-Antarctic caterpillar, *Embryonopsis halticella* (Lepidoptera: Yponomeutidae). Journal of Insect Physiology 44, 615–628.
Leather, S.R., Walters, K.F.A., Bale, J.S., 1993. The Ecology of Insect Overwintering. Cambridge University Press, Cambridge.
Lee, C.E., Remfert, J.L., Gelembiuk, G.W., 2003. Evolution of physiological tolerance and performance during freshwater invasion. Integrative and Comparative Biology 43, 439–449.
McGeoch, M.A., le Roux, P.C., Hugo, E.C., Chown, S.L., 2006. Species and community responses to short-term climate manipulation: microarthropods in the sub-Antarctic. Austral Ecology 31, 719–731.
Nedvěd, O., Lavy, D., Verhoef, H.A., 1998. Modelling the time–temperature relationship in cold injury and effect of high-temperature interruptions on survival in a chill-sensitive Collembolan. Functional Ecology 12, 816–824.
Pryor, M.E., 1962. Some environmental features of Hallett station, Antarctica, with special reference to soil arthropods. Pacific Insects 4, 681–728.
Ramløv, H., 2000. Aspects of natural cold tolerance in ectothermic animals. Human Reproduction 15, 26–46.
Renault, D., Nedvěd, O., Hervant, F., Vernon, P., 2004. The importance of fluctuating thermal regimes for repairing chill injuries in the tropical beetle *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) during exposure to low temperature. Physiological Entomology 29, 139–145.
Ryan, P.G., Watkins, B.P., 1989. The influence of physical factors and ornithogenic products on plant and arthropod abundance at an inland nunatak group in Antarctica. Polar Biology 10, 151–160.
Salt, R.W., 1966. Effect of cooling rate on the freezing temperatures of supercooled insects. Canadian Journal of Zoology 44, 655–659.
Sinclair, B.J., 2001. Field ecology of freeze tolerance: interannual variation in cooling rates, freeze–thaw and thermal stress in the microhabitat of the alpine cockroach *Celatoblatta quinquemaculata*. Oikos 93, 286–293.
Sinclair, B.J., Chown, S.L., 2002. Haemolymph osmolality and thermal hysteresis activity in 17 species of arthropods from sub-Antarctic Marion Island. Polar Biology 25, 928–933.
Sinclair, B.J., Chown, S.L., 2005a. Climatic variability and hemispheric differences in insect cold tolerance: support from southern Africa. Functional Ecology 19, 214–221.
Sinclair, B.J., Chown, S.L., 2005b. Deleterious effects of repeated cold exposure in a freeze-tolerant sub-Antarctic caterpillar. Journal of Experimental Biology 208, 869–879.
Sinclair, B.J., Sjursen, H., 2001. Cold tolerance of the Antarctic springtail *Gomphiocephalus hogsoni* (Collembola, Hypogastruridae). Antarctic Science 13, 271–279.
Sinclair, B.J., Klok, C.J., Scott, M.B., Terblanche, J.S., Chown, S.L., 2003a. Diurnal variation in supercooling points of three species of Collembola from Cape Hallett, Antarctica. Journal of Insect Physiology 49, 1049–1061.
Sinclair, B.J., Vernon, P., Klok, C.J., Chown, S.L., 2003b. Insects at low temperatures: an ecological perspective. Trends in Ecology and Evolution 18, 257–262.
Sinclair, B.J., Terblanche, J.S., Scott, M.B., Blatch, G.L., Klok, C.J., Chown, S.L., 2006. Environmental physiology of three species of Collembola at Cape Hallett, North Victoria Land, Antarctica. Journal of Insect Physiology 52, 29–50.
Slabber, S., Chown, S.L., 2004. Thermal tolerance and cold hardiness strategy of the sub-Antarctic psocid *Antarctopsocus jeanneli* Badonnel. Polar Biology 28, 56–61.
Slabber, S., Chown, S.L., 2005. Differential responses of thermal tolerance to acclimation in the sub-Antarctic rove beetle *Halmaeusa atriceps*. Physiological Entomology 30, 195–204.
Smith, V.R., 2002. Climate change in the sub-Antarctic: an illustration from Marion Island. Climatic Change 52, 345–357.
Sømme, L., 1978. Cold-hardiness of *Cryptopygus antarcticus* (Collembola) from Bouvetøya. Oikos 31, 94–97.
Sømme, L., 1982. Supercooling and winter survival in terrestrial arthropods. Comparative Biochemistry and Physiology A 73, 519–543.
Sømme, L., 1986a. Terrestrial arthropods of Bouvetøya. Polar Research 4, 231–233.

Sømme, L., 1986b. Ecology of *Cryptopygus sverdrupi* (Insecta: Collembola) from Dronning Maud Land, Antarctica. Polar Biology 6, 179–184.

Sømme, L., 1999. The physiology of cold hardiness in terrestrial arthropods. European Journal of Entomology 96, 1–10.

Sømme, L., Block, W., 1982. Cold hardiness of Collembola at Signy Island, maritime Antarctic. Oikos 38, 168–176.

Stachowicz, J.J., Terwin, J.R., Whitlatch, R.B., Osman, R.W., 2002. Linking climate change and biological invasions: ocean warming facilitates nonindigenous species invasions. Proceedings of the National Academy of Sciences of the USA 99, 15497–15500.

Stevens, M.I., Greenslade, P., Hogg, I.D., Sunnucks, P., 2006a. Southern hemisphere springtails: could any have survived glaciation of Antarctica? Molecular Biology and Evolution 23, 874–882.

Stevens, M.I., Fjellberg, A., Greenslade, P., Hogg, I.D., Sunnucks, P., 2006b. Redescription of the Antarctic springtail *Desoria klovstadi* using morphological and molecular evidence. Polar Biology 29, 820–830.

Terblanche, J.S., Klok, C.J., Krafsur, E.S., Chown, S.L., 2006. Phenotypic plasticity and geographic variation in thermal tolerance and water loss of the tsetse *Glossina pallidipes* (Diptera: Glossinidae): implications for distribution modelling. American Journal of Tropical Medicine and Hygiene 74, 786–794.

Usher, M.B., Booth, R.G., 1986. Arthropod communities in a maritime Antarctic moss-turf habitat: multiple scales of pattern in the mites and Collembola. Journal of Animal Ecology 55, 155–170.

Van der Woude, H.A., Verhoef, H.A., 1986. A comparative study of winter survival of temperate Collembola. Ecological Entomology 11, 333–340.

Van der Woude, H.A., Verhoef, H.A., 1988. Reproductive diapause and cold hardiness in temperate Collembola *Orchesella cincta* and *Tomocerus minor*. Journal of Insect Physiology 34, 387–392.

Walther, G.-R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., Fromentin, J.-M., Hoegh-Guldberg, O., Bairlein, F., 2002. Ecological responses to recent climate change. Nature 416, 389–395.

Wise, K.A.J., 1967. Collembola (Springtails). In: Gressitt, J.L. (Ed.), Entomology of Antarctica. American Geophysical Union, Washington, DC, pp. 123–148.

Worland, M.R., 2005. Factors that influence freezing in the sub-Antarctic springtail *Tullbergia antarctica*. Journal of Insect Physiology 51, 881–894.

Worland, M.R., Convey, P., 2001. Rapid cold hardening in Antarctic microarthropods. Functional Ecology 15, 515–524.

Worland, M.R., Leinaas, H.P., Chown, S.L., 2006. Supercooling point frequency distributions in Collembola are affected by moulting. Functional Ecology 20, 323–329.